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Patent No.: IBIS-0369
Title: MASS SPECTROMETRIC METHODS FOR
BIOMOLECULAR SCREENING
Inventors: Stanley T. Crooke, Richard Griffey and
Steven Hofstadler
Atty: Paul K. Legaard - Telephone: 215 568 3100
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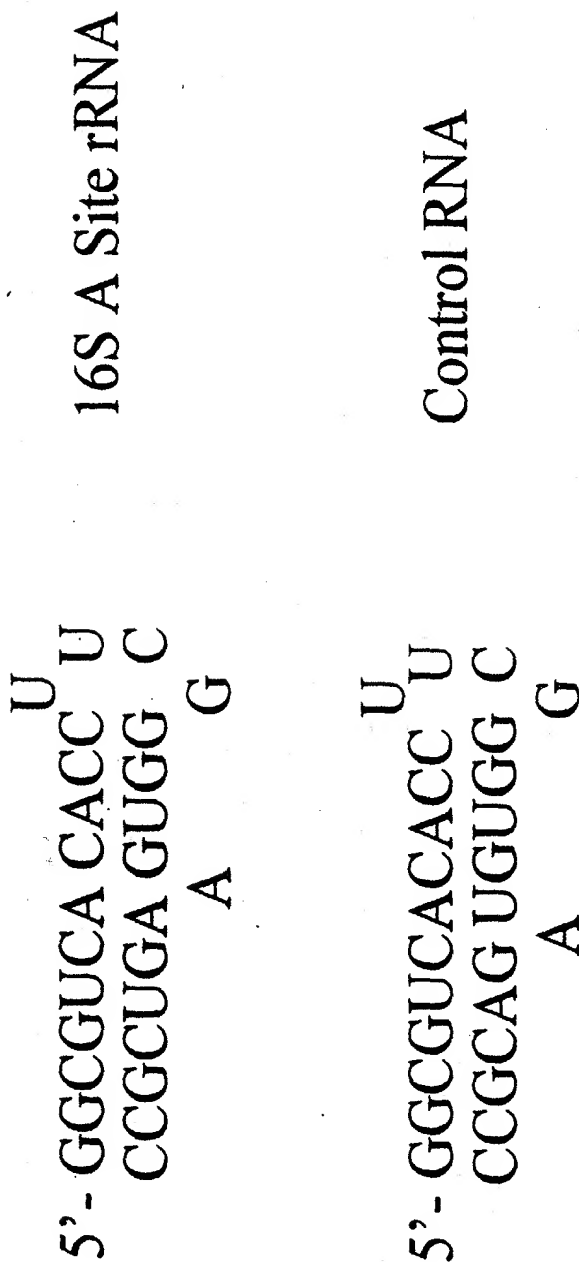


Figure 1. Sequence and structure of 27mer RNA target

0584434 051901 106790 1748860

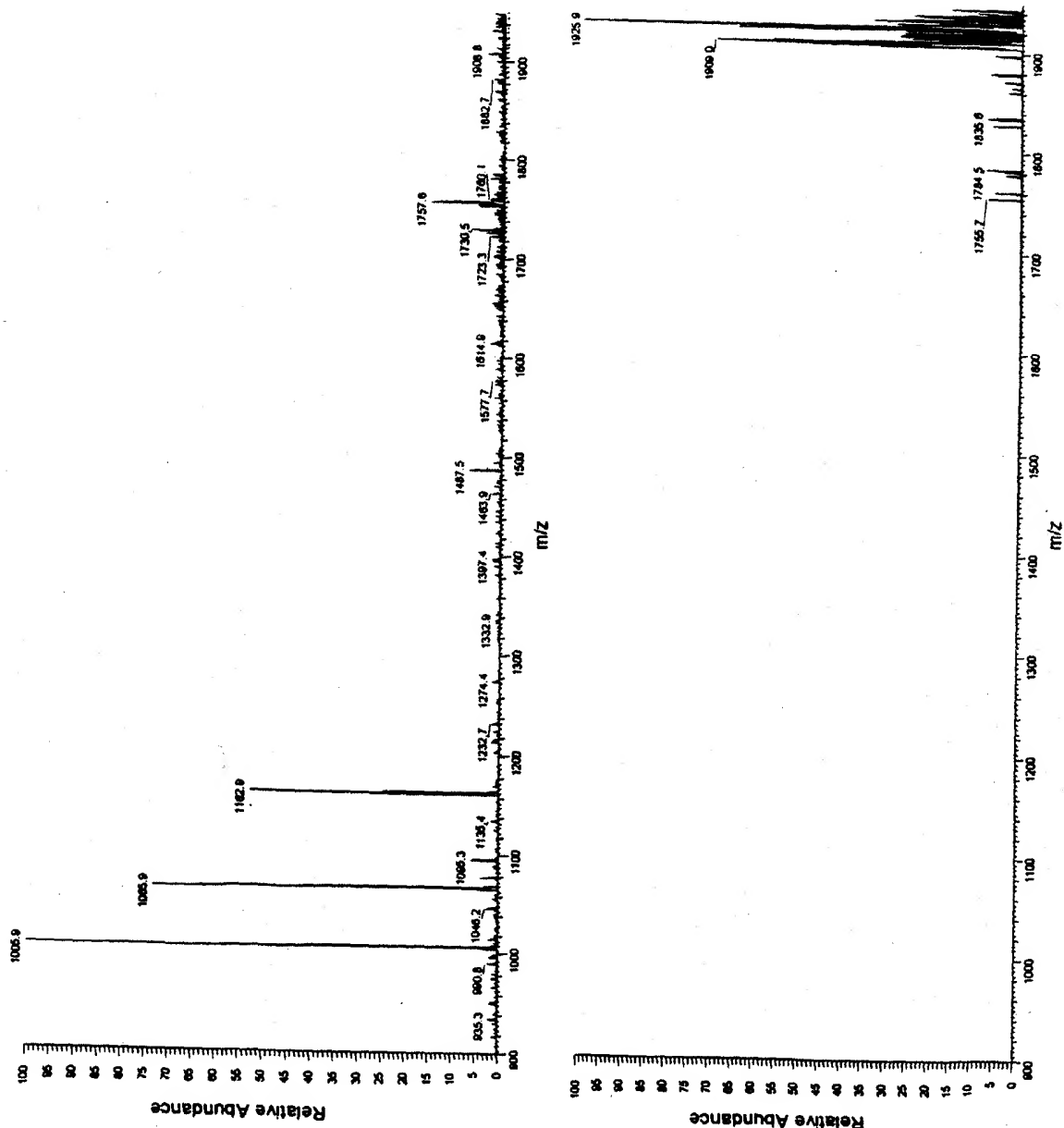


Figure 2. MS/MS of control RNA/DNA (upper); control + paromomycin (lower)

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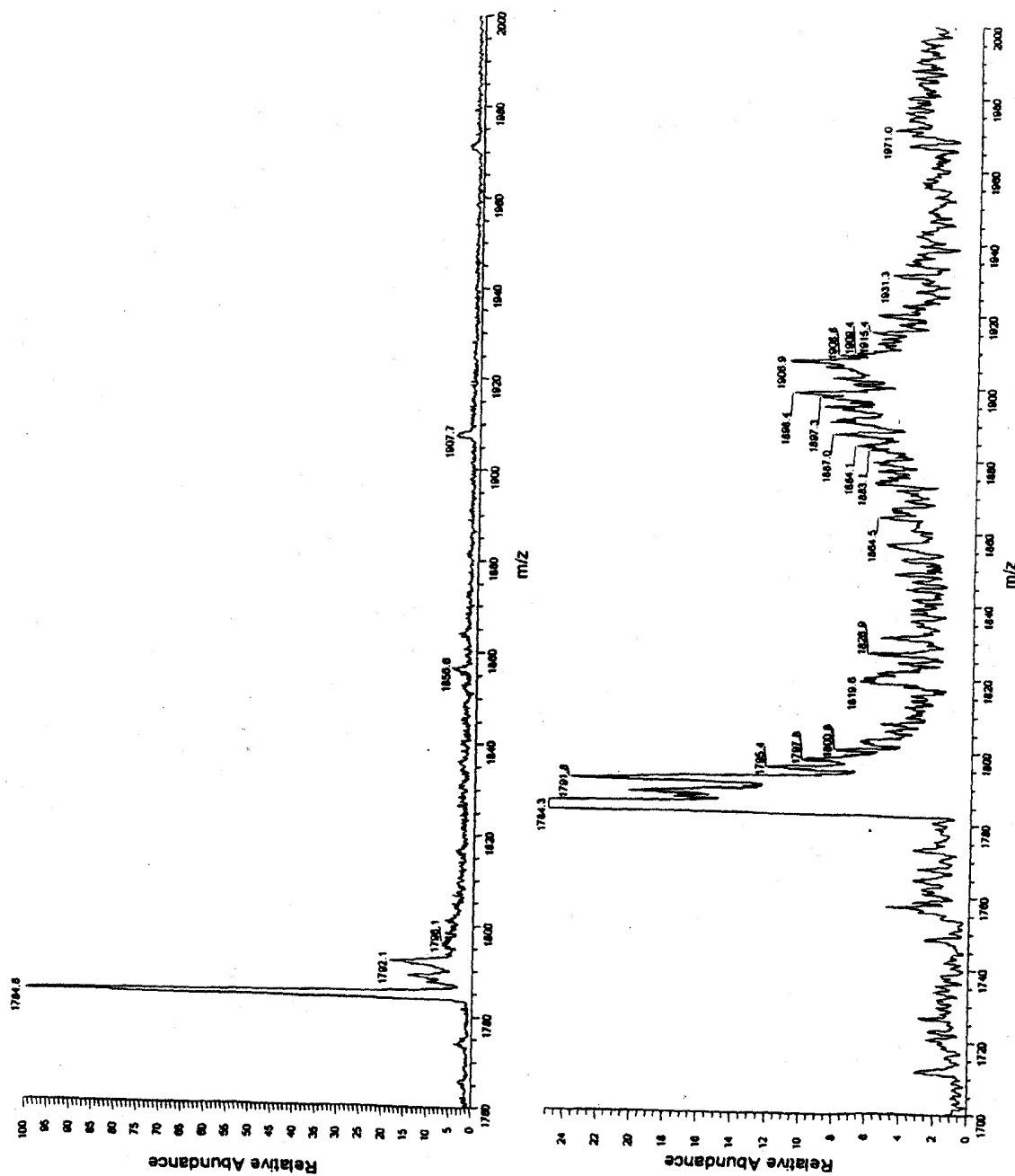


Figure 3. RNA/DNA chimera+paramomycin (upper); chimera+library (lower)

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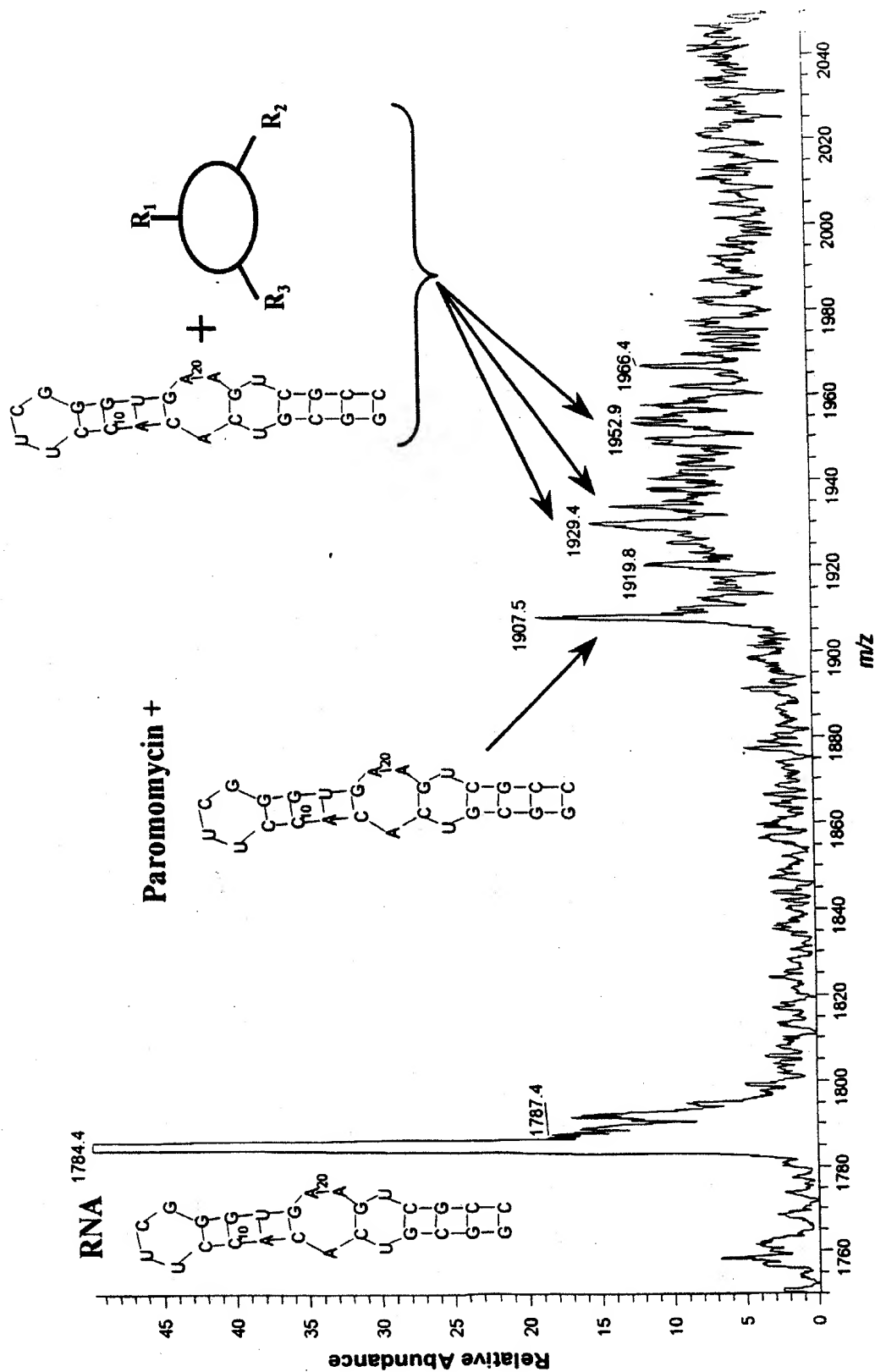


Figure 5. ESI-MS of RNA/DNA chimera bound to paromomycin and library

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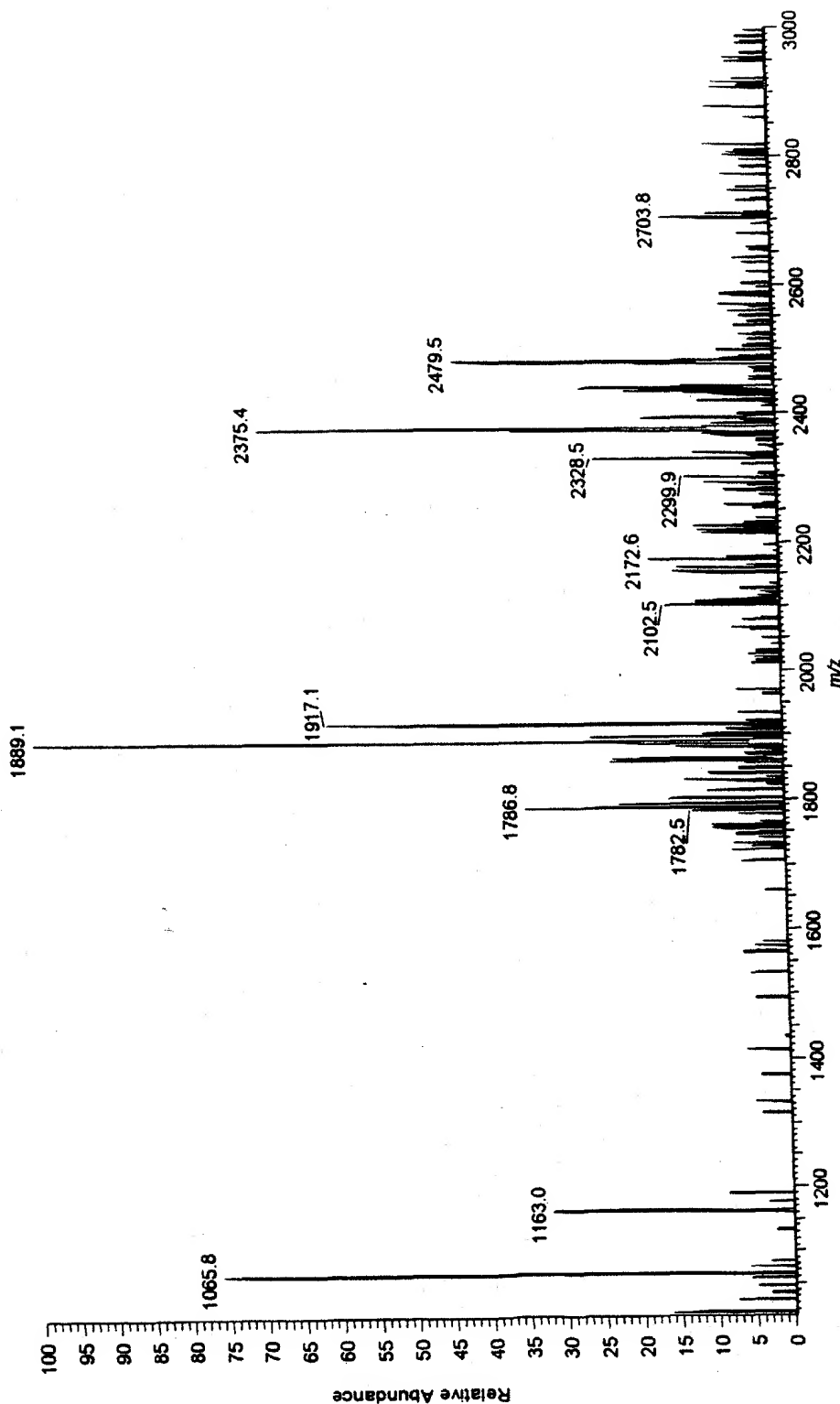


Figure 6. MS/MS of RNA/DNA chimera + compound with mass 665.1 not bound at the A-site

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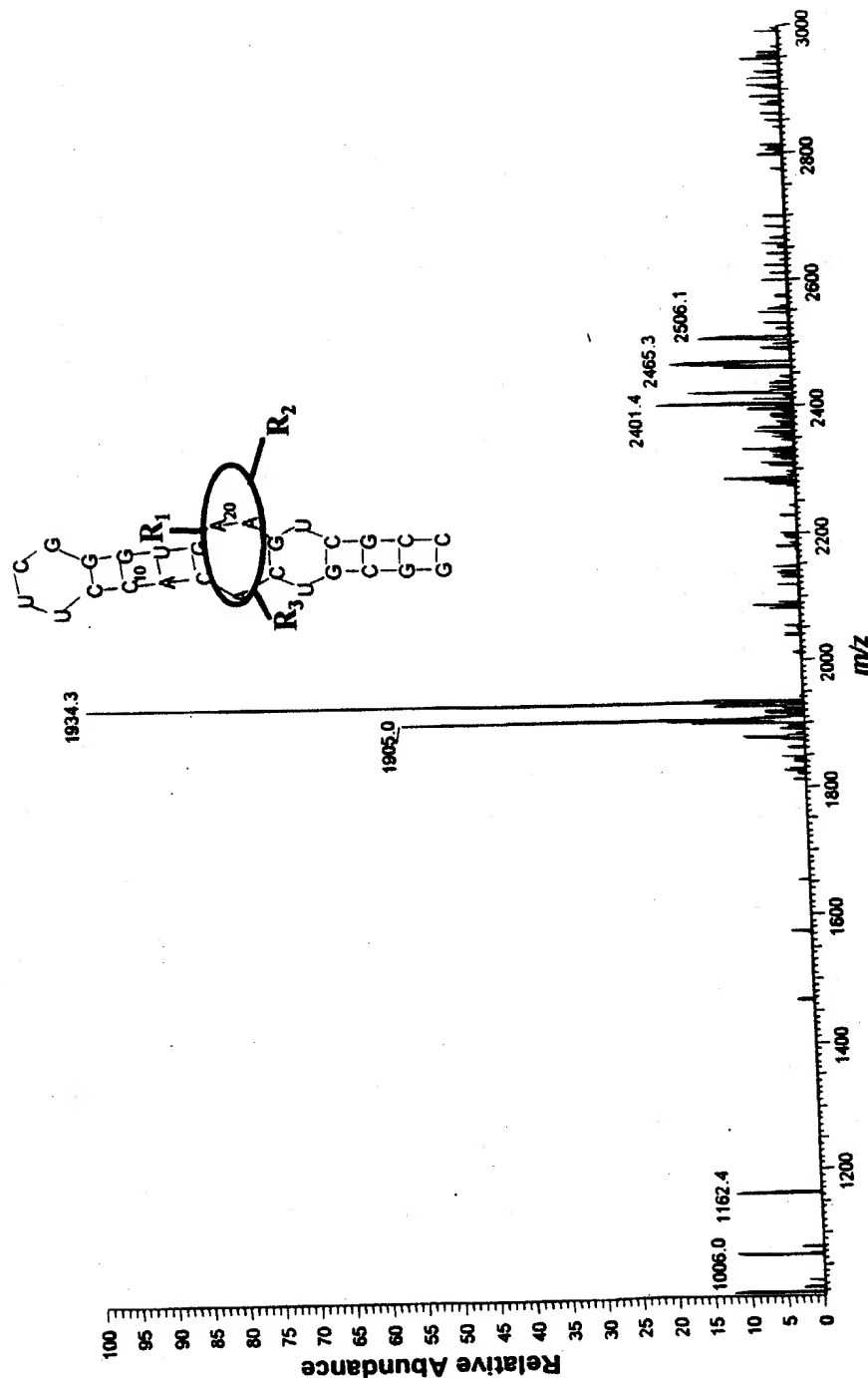


Figure 7. MS-MS analysis of member bound to RNA/DNA chimera at the A-Site



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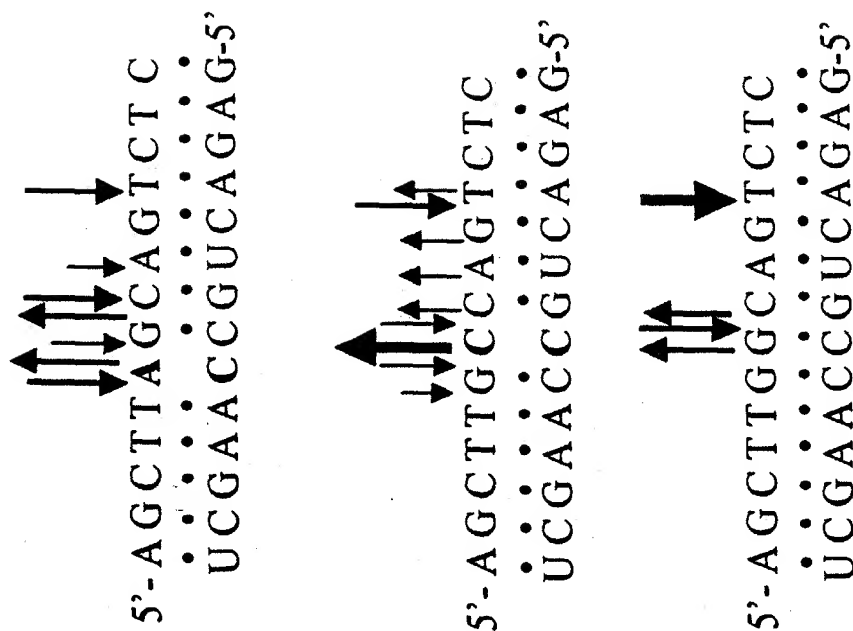


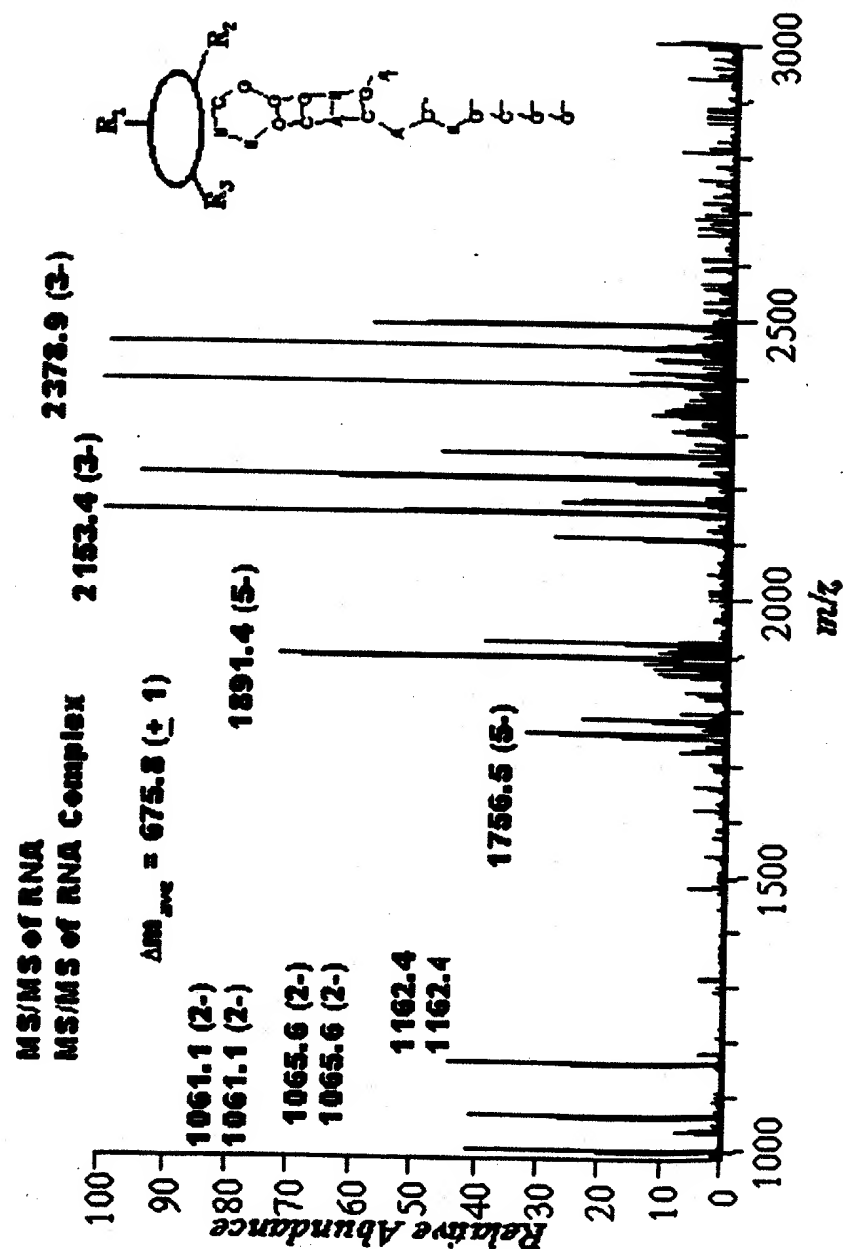
Figure 9. MS Fragmentation of DNA:RNA duplexes

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MASS Analysis of Binding Location

non-A site binder

FIGURE 10



MASS Analysis of Binding Location

non-A site binder

FIGURE 11

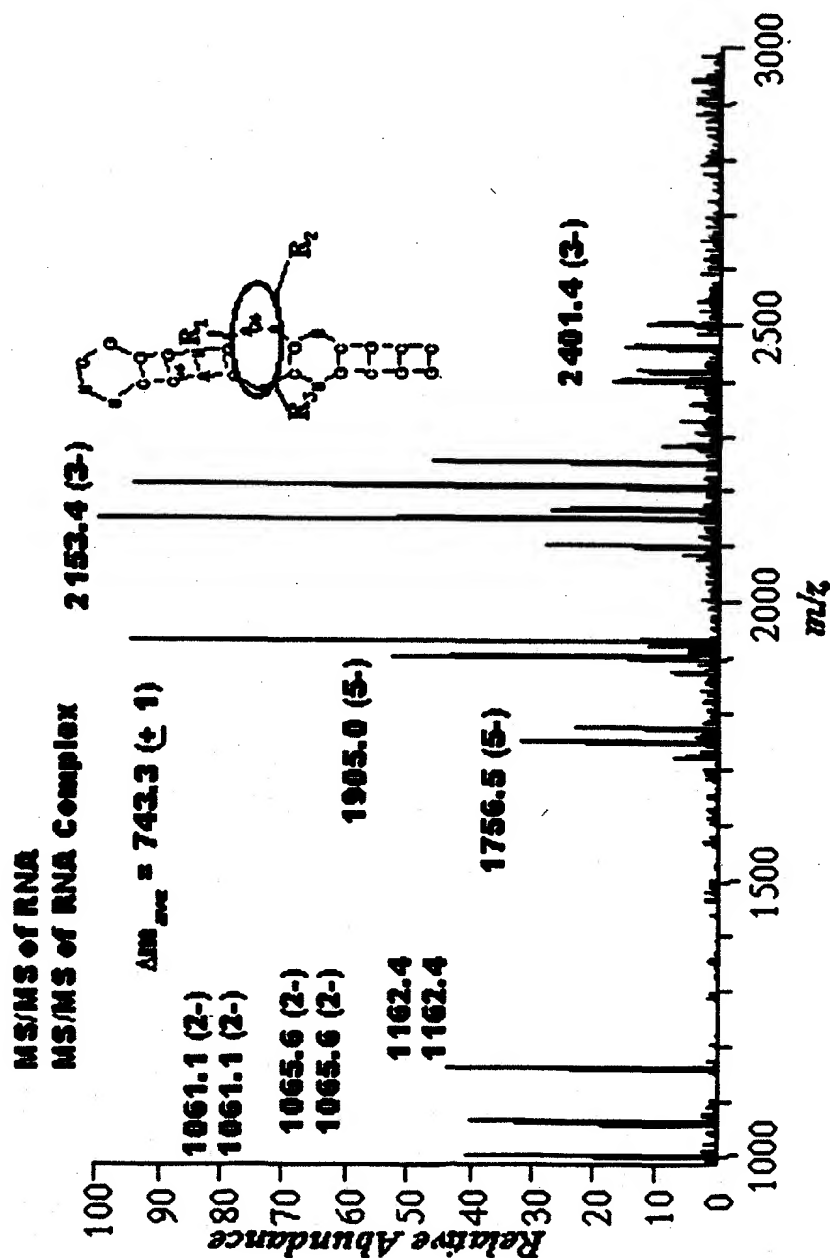
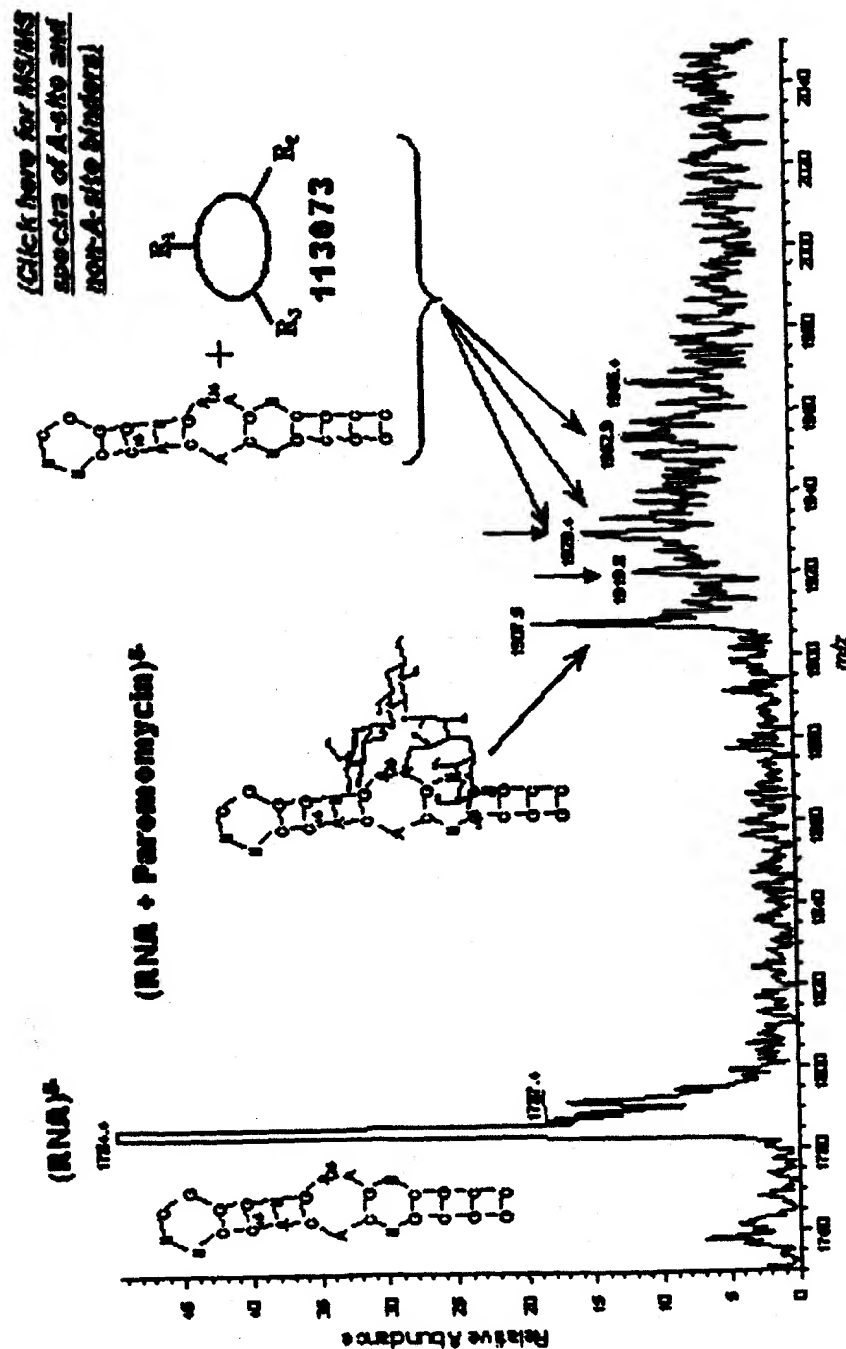


FIGURE 12

MASS analysis of 16S A site RNA plus 216 member library (performed on quadrupole ion trap)

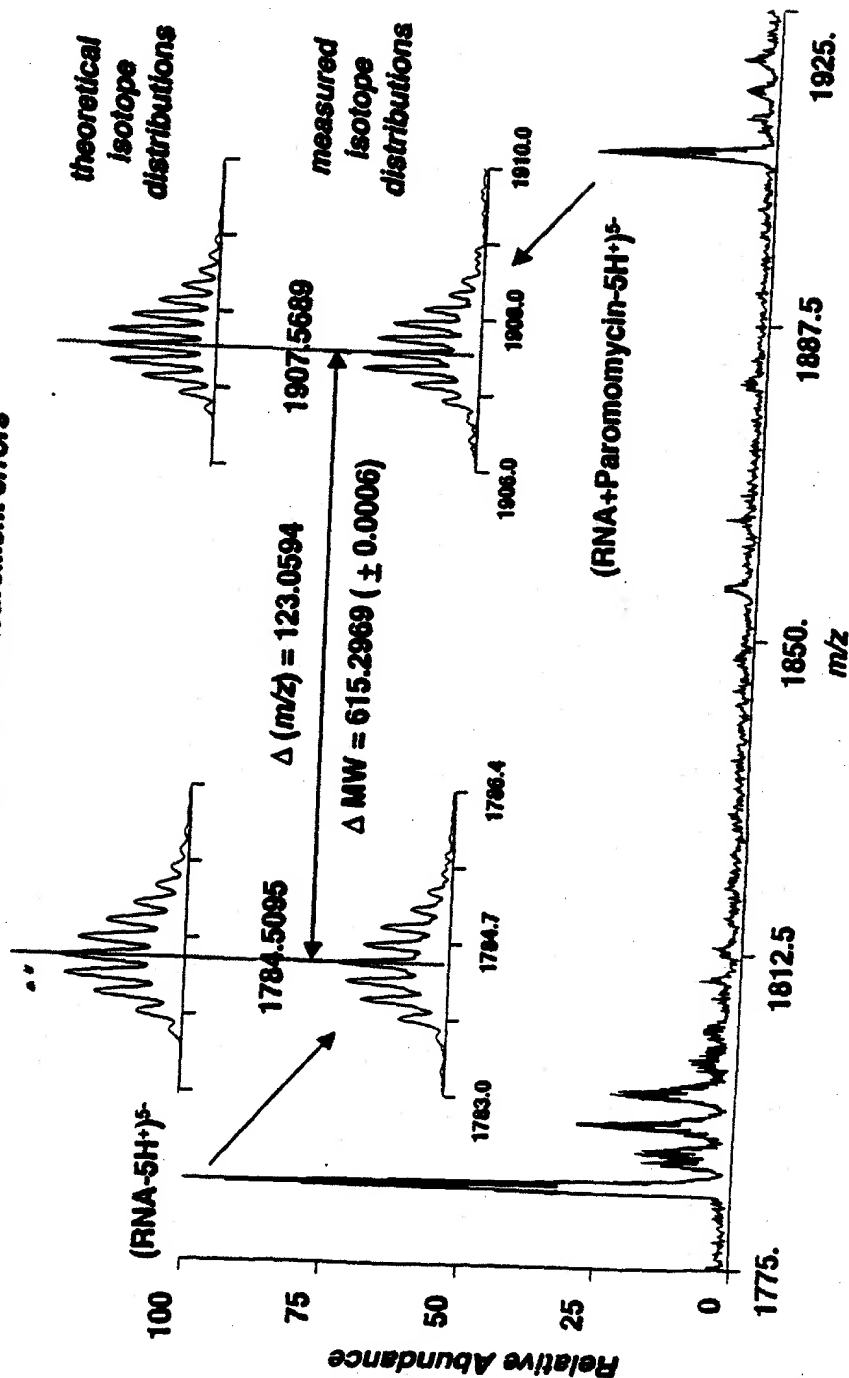
FIGURE 12



High Precision ESI-FTICR Mass Measurement of 16S A site RNA/Paromomycin Complex

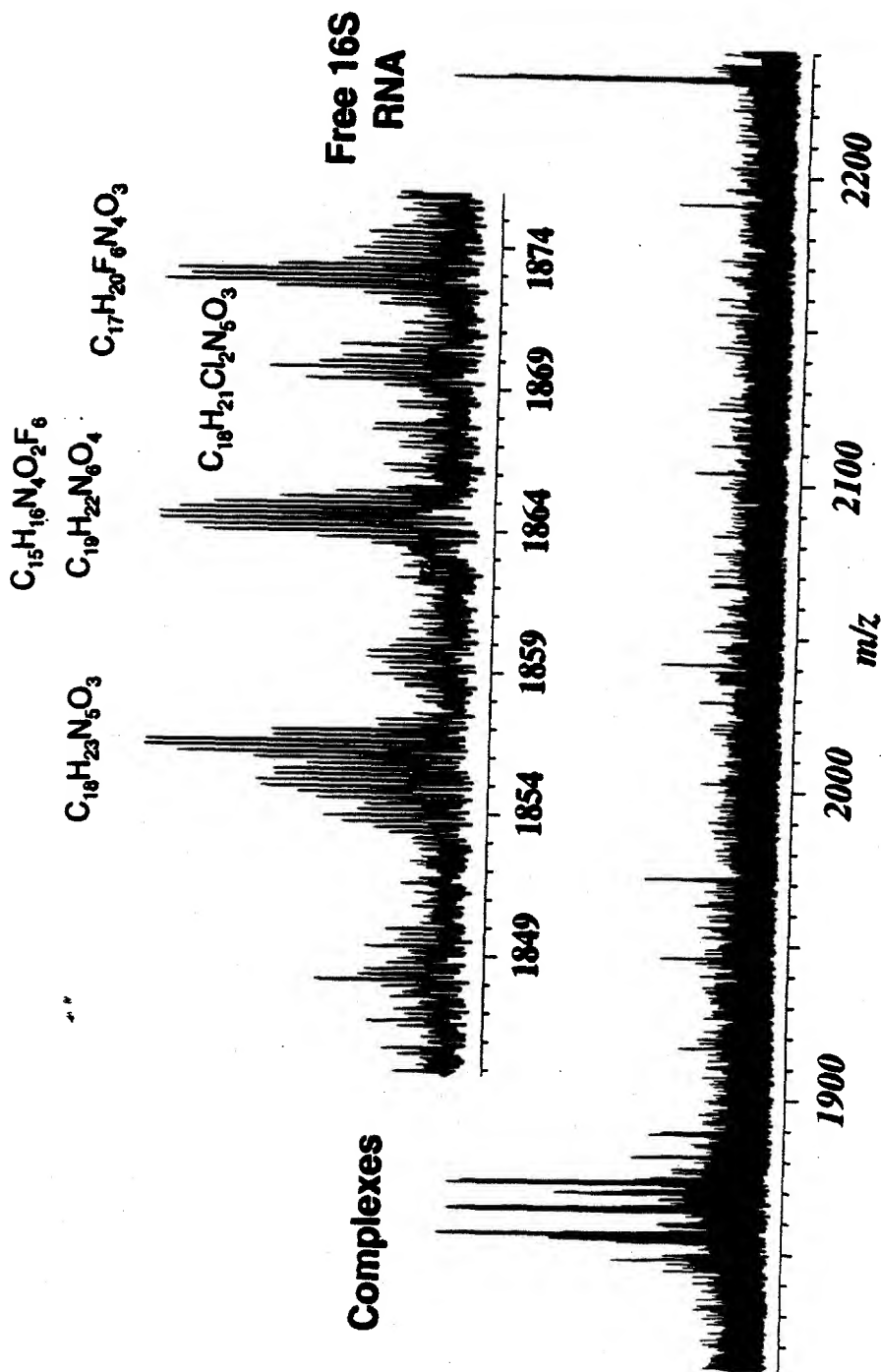
Figure 13

use of unbound RNA as internal mass standard
 provides low ppm mass measurement errors



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Figure 14
MASS of 60-Member Ibis Library Against 16S A-site RNA



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Figure 15
MASS of 60-member Library against 16S A-site Model

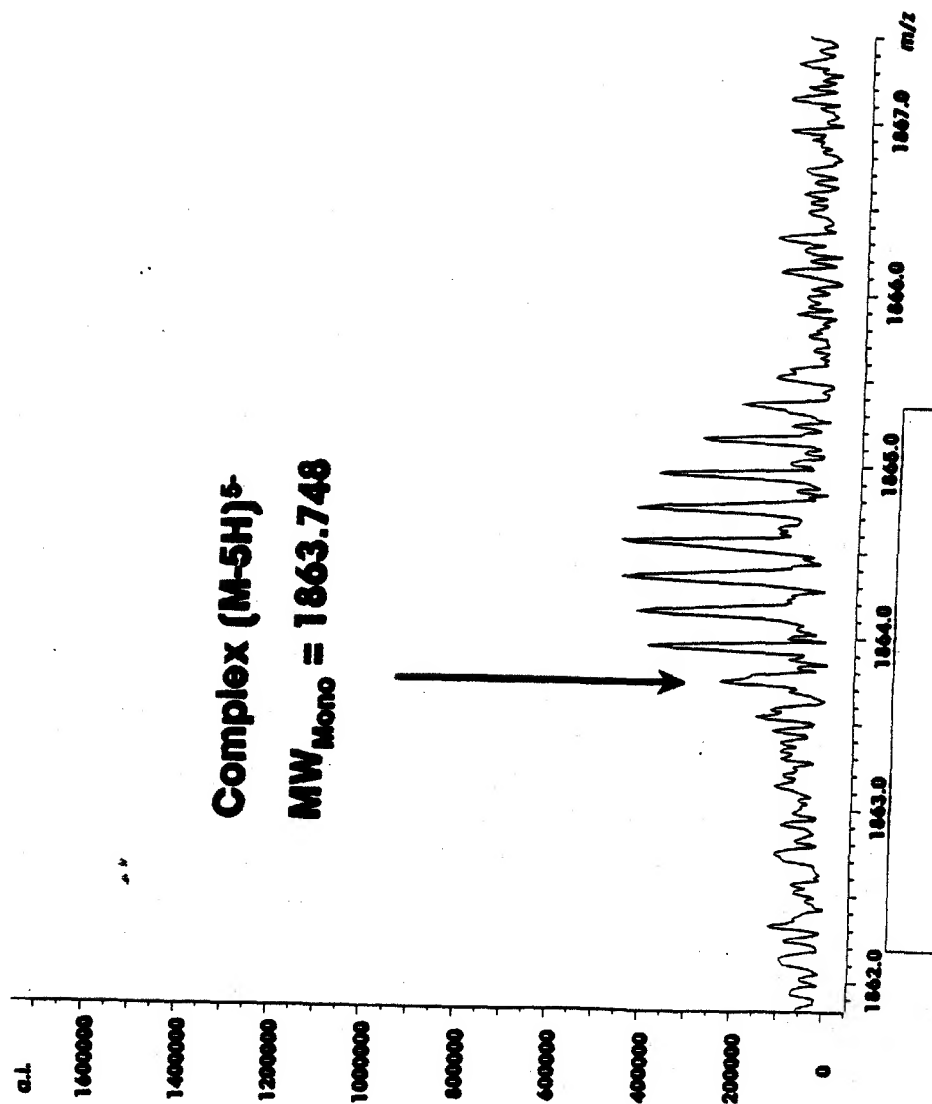


Figure 16

FT-ICR MS of Starting Library

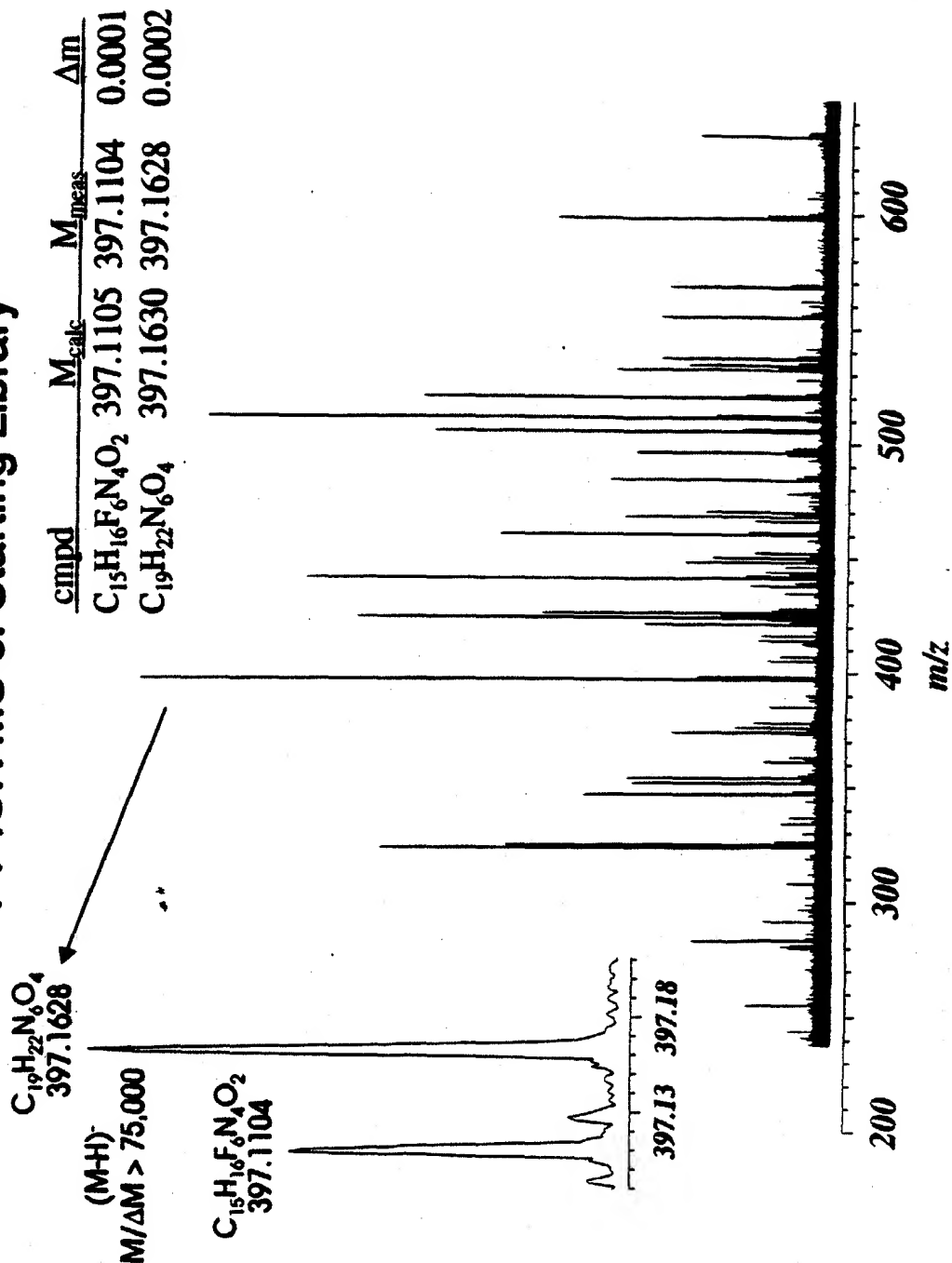


Figure 17
Compound Identification from a 60-member
Combinatorial library with MASS

Complex M_{meas}	9320.300\pm.009 Da
RNA M_{meas}	8922.189\pm.009
ΔM	398.111\pm.009 Da



Figure 18

Elemental Composition Constraints

Measured Mass: 615.2969
 Mass Tolerance: 1.0 ppm
 Charge: 0

Element	Min. atoms	Max. atoms
¹² C	12	30
¹ H	23	60
¹⁶ O	7	20
¹⁴ N	3	20

Possible Elemental Compositions:

Calc. Mass (amu)	Error (ppm)	Molecular Formula
615.296291	0.98	¹⁶ O ₄ ¹⁴ N ₁₉ ¹² C ₂₁ ¹ H ₃₃
615.296298	0.98	¹⁶ O ₉ ¹⁴ N ₁₂ ¹² C ₂₂ ¹ H ₃₉
615.296305	0.97	¹⁶ O ₁₄ ¹⁴ N ₅ ¹² C ₂₃ ¹ H ₄₅
615.296808	0.15	¹⁶ O ₁₅ ¹⁴ N ₁₇ ¹² C ₈ ¹ H ₄₁
615.296815	0.14	¹⁶ O ₂₀ ¹⁴ N ₁₀ ¹² C ₉ ¹ H ₄₇

Further constrain by
 elemental
 composition of
 "letters"
 unintended
 products...

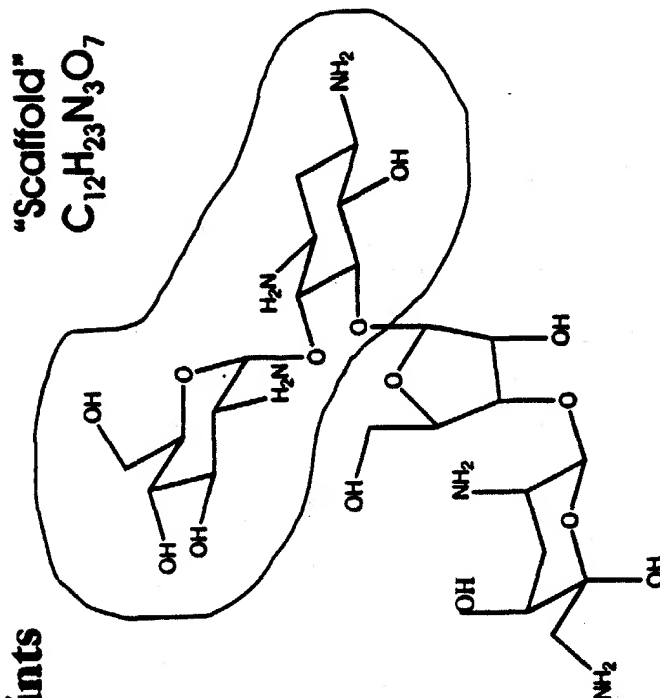
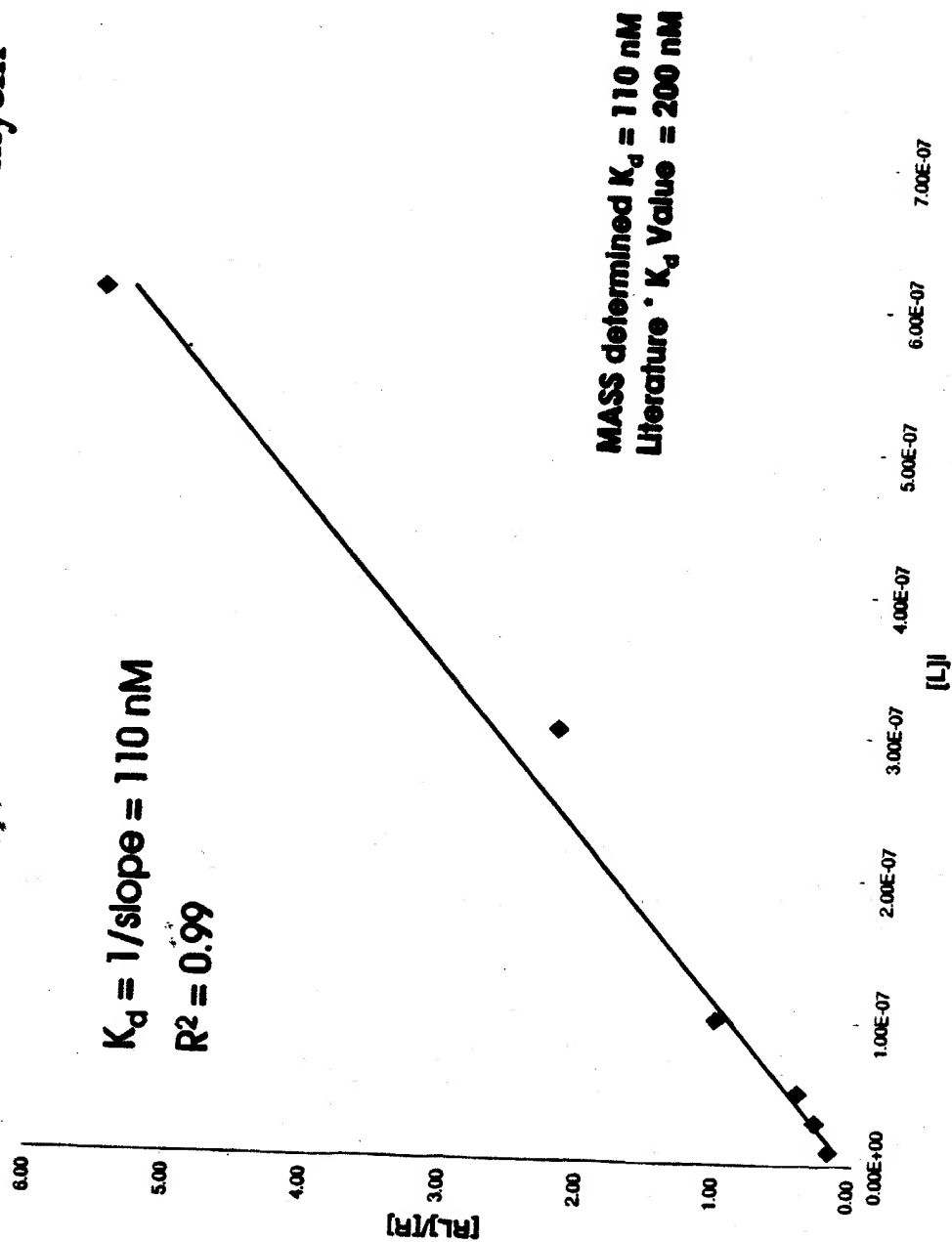
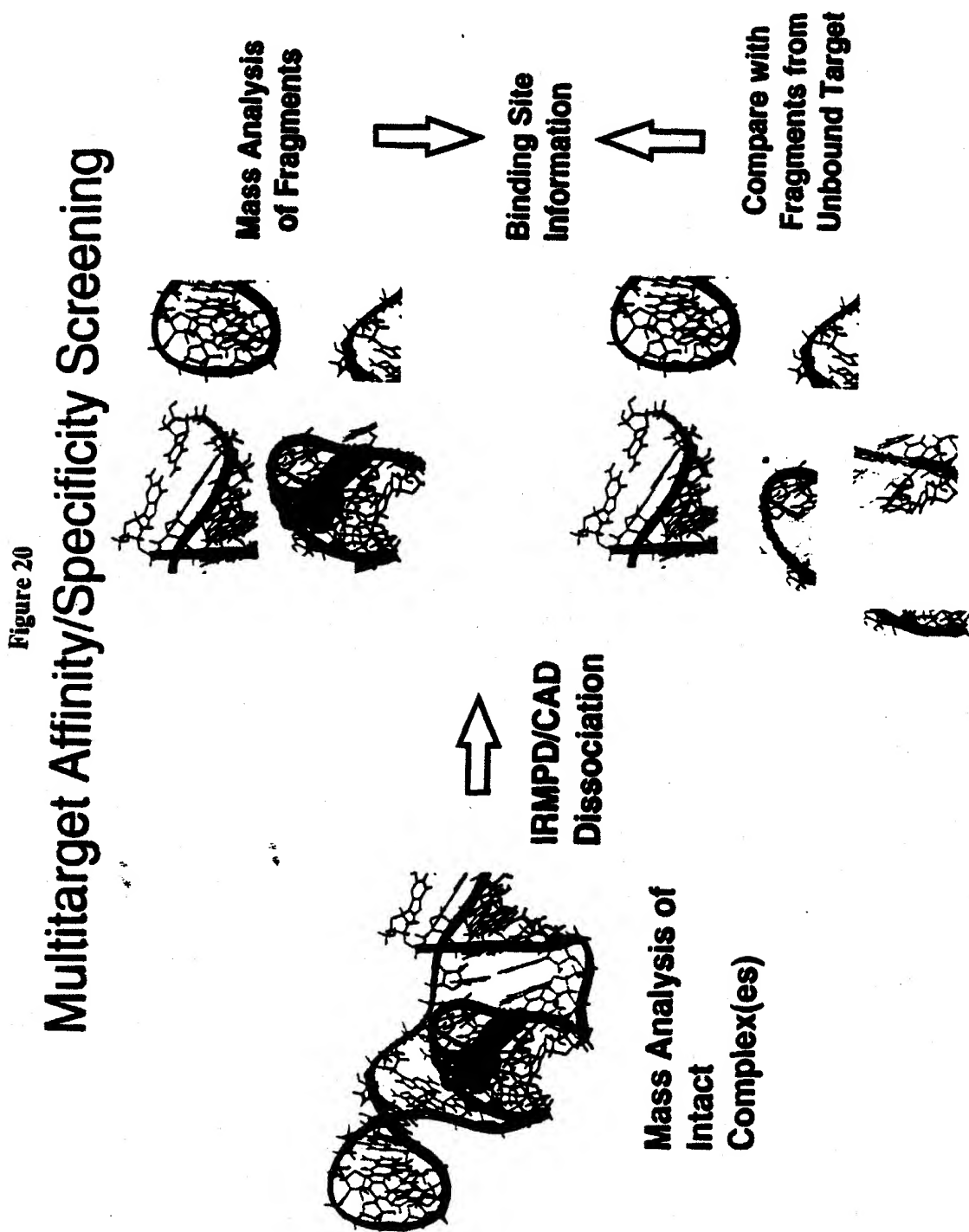


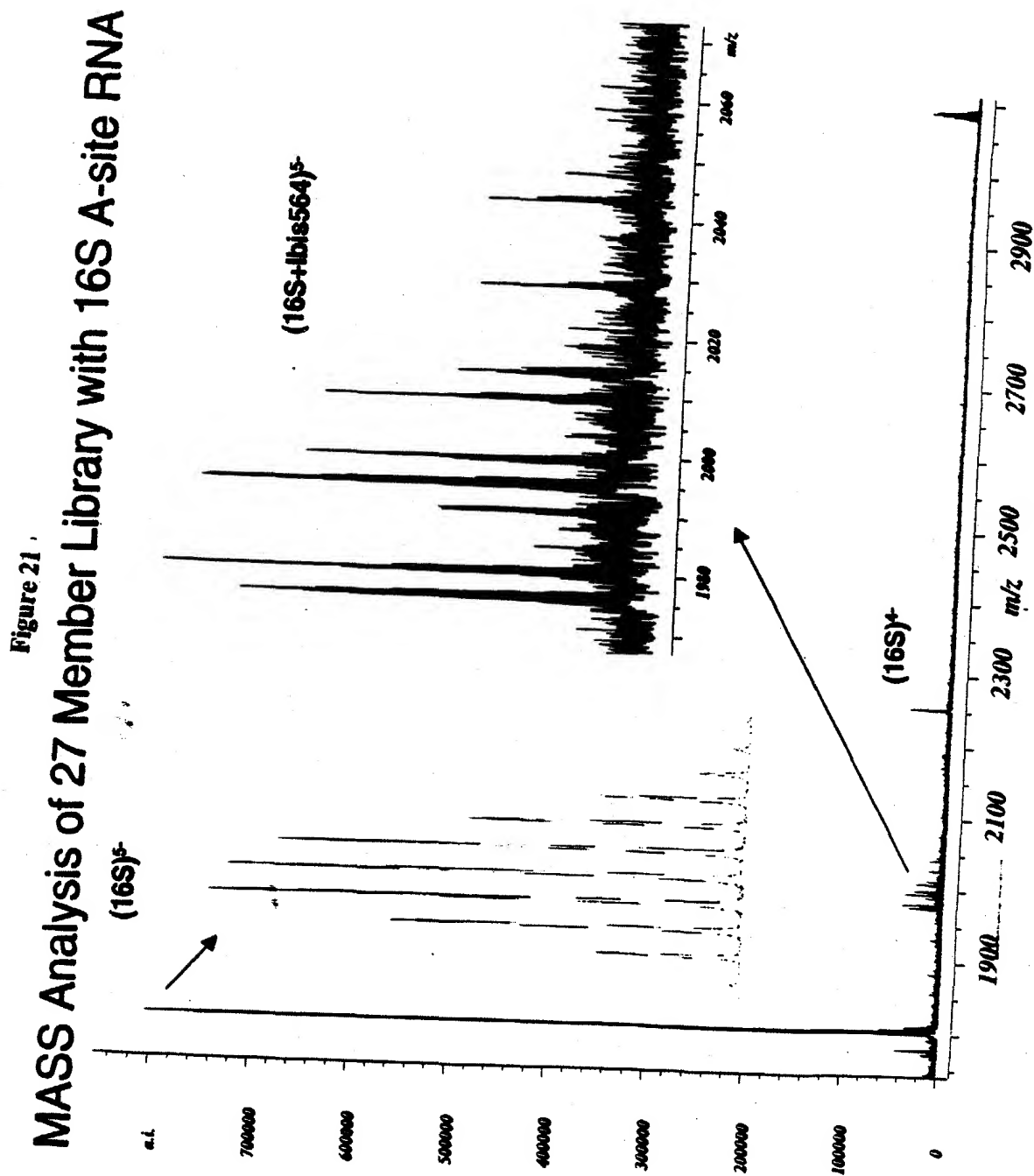
Figure 19
 MASS K_d determination for 16S-Paromomycin





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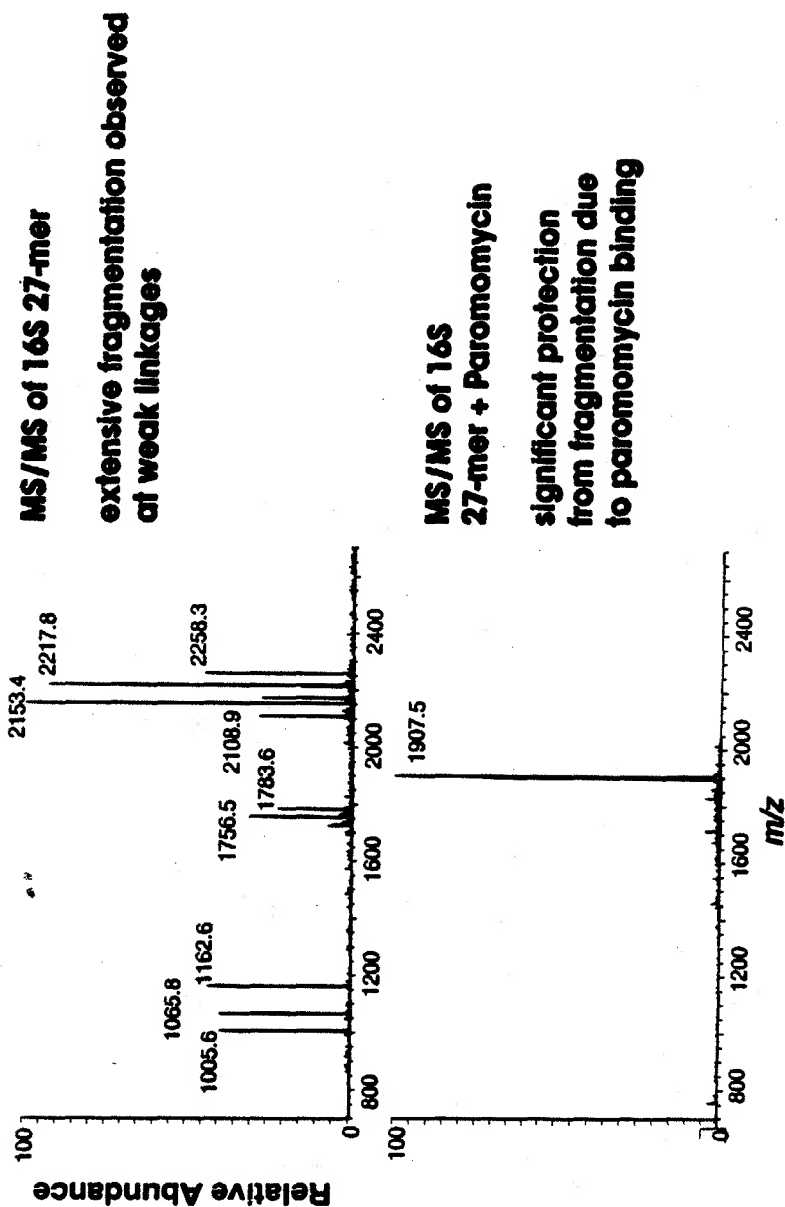
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Figure 22

MASS Protection Assay



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Figure 23

MASS Protection Assay

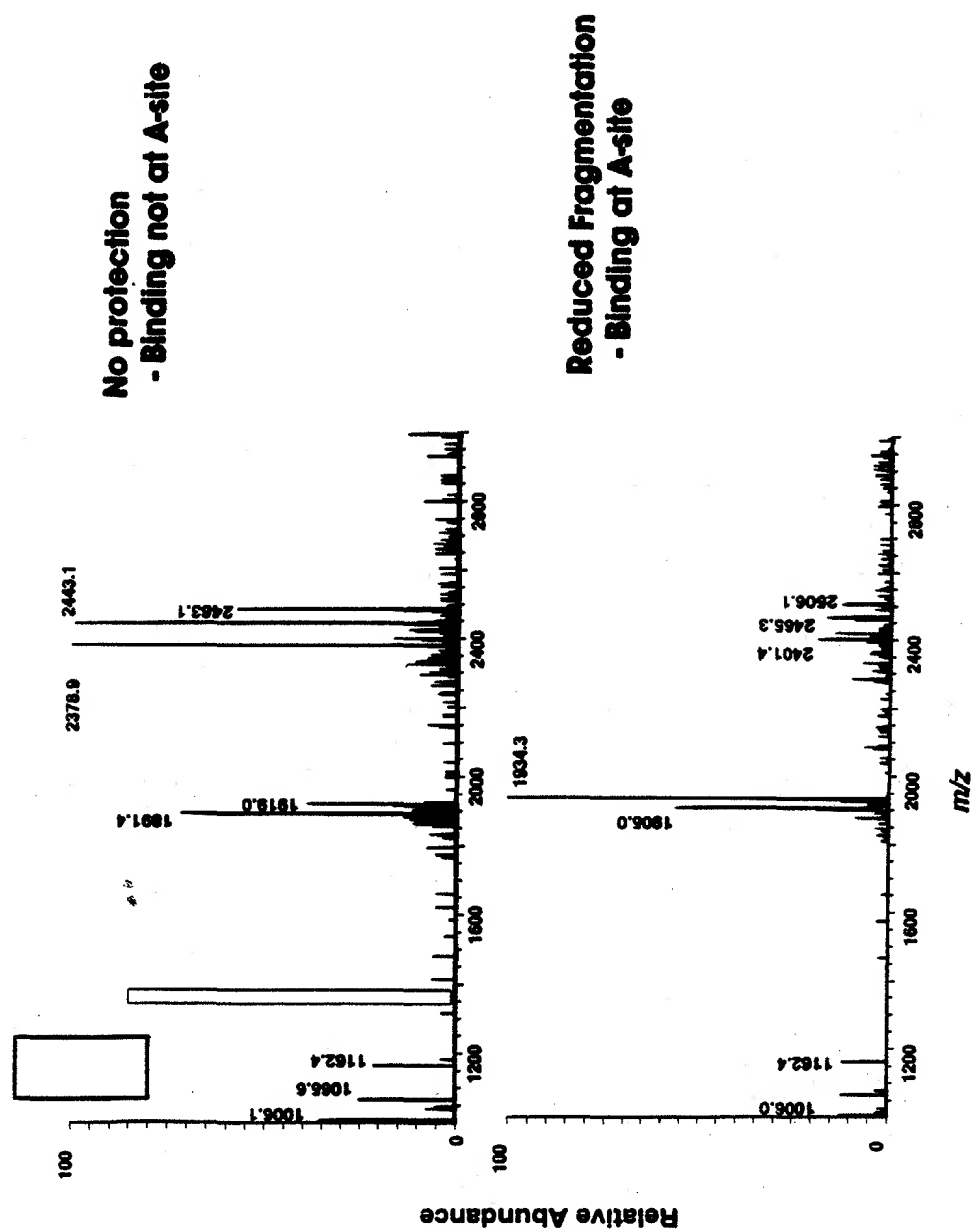
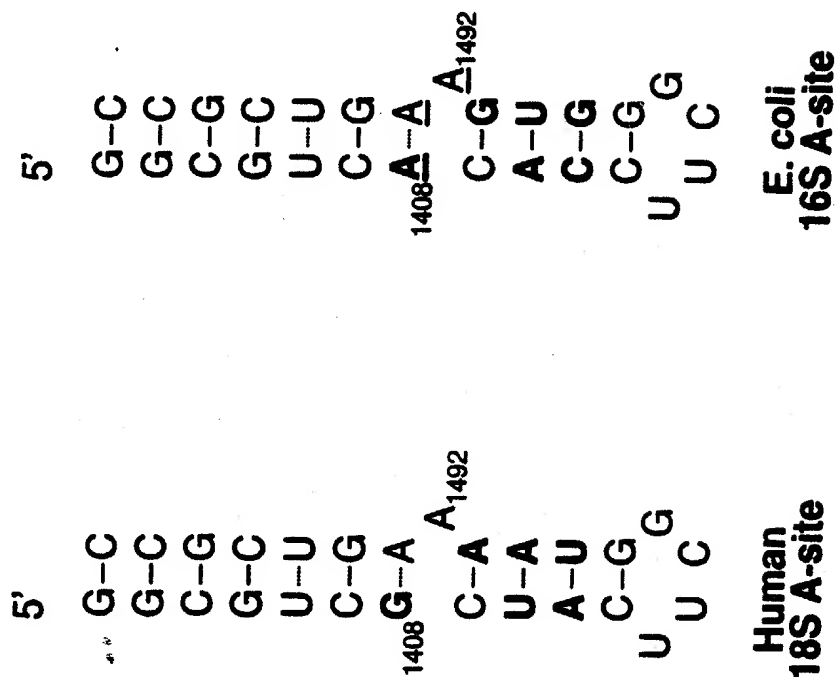


Figure 24
Eukaryotic and Prokaryotic A-Sites
 Aminoglycoside antibiotics bind to
 A-site of decoding region in 16S RNA



Δ MW = 15.011 Da

FIGURE 25

Figure 25
Neutral Mass Tag Does Not Affect Ligand Binding

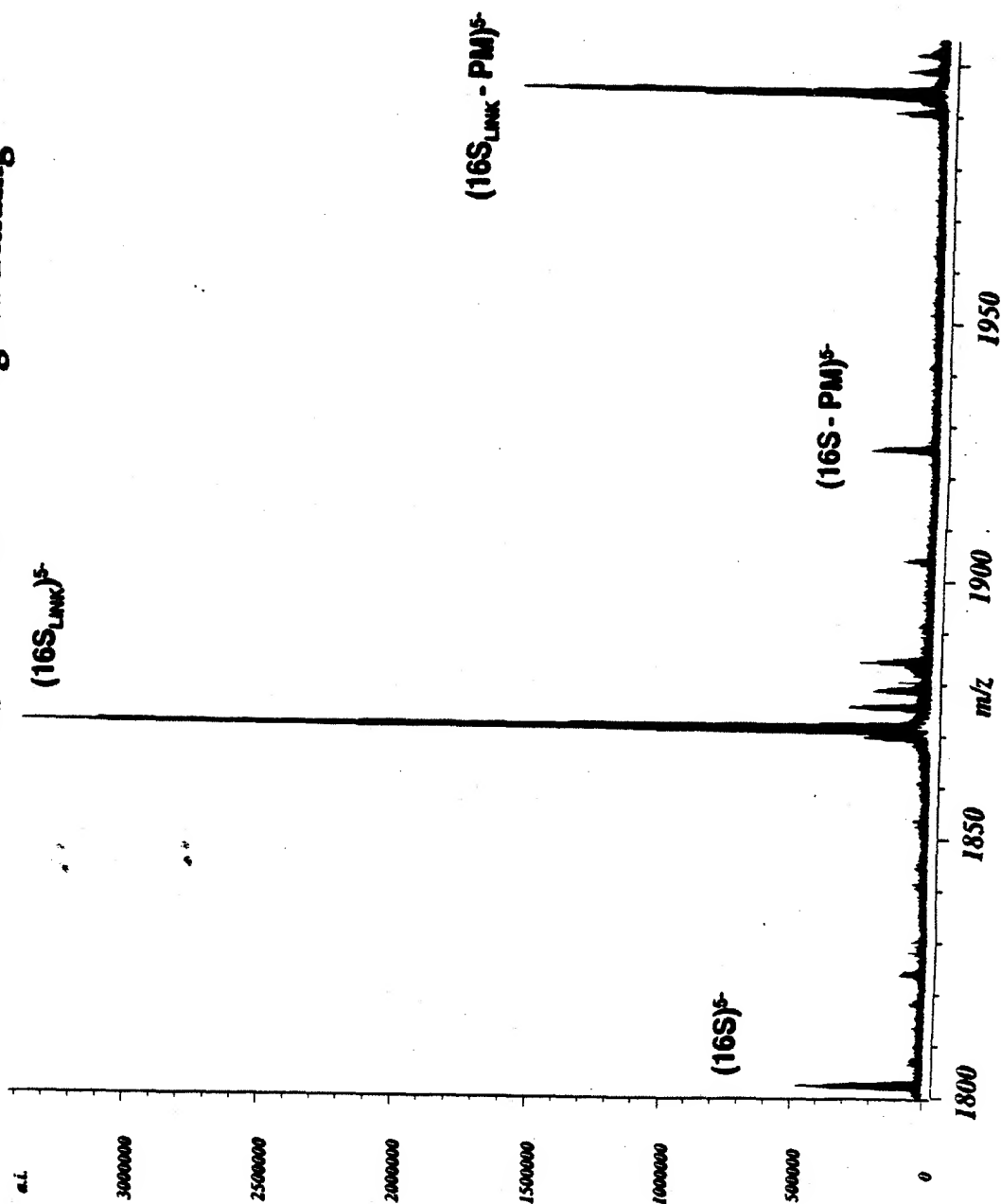
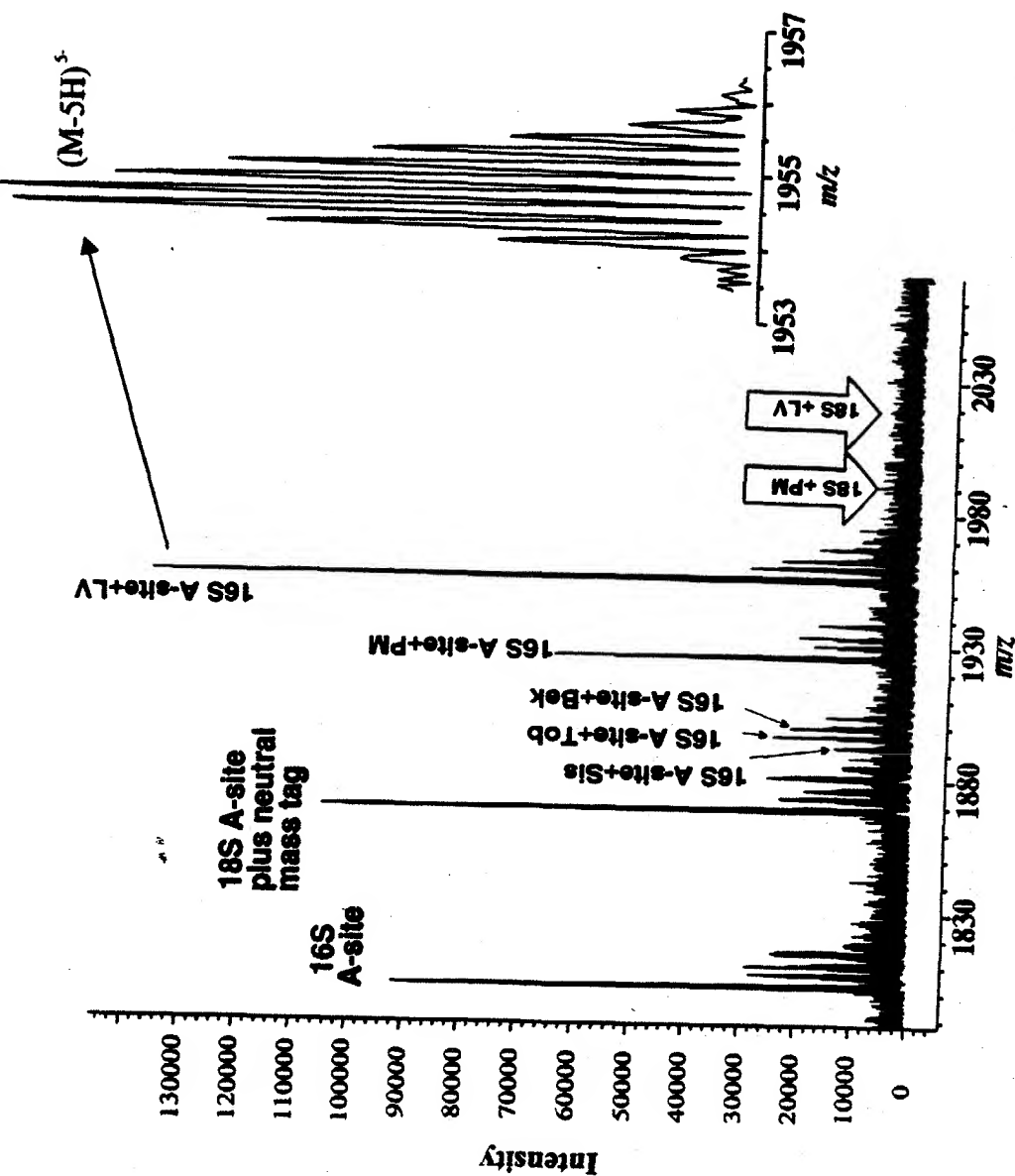


Figure 26

Simultaneous Screening of 16S A-site and 18S A-site Model RNAs Against Aminoglycoside Mixture



5'

G-C

G-C

C-G

G-C

U-U

C-G

¹⁴⁰⁸Δ-Δ

C-G ^{Δ1408}

A-U

C-G

U C-G G

U C

R Δ = adenosine

C Δ = deoxyadenosine

Figure 28

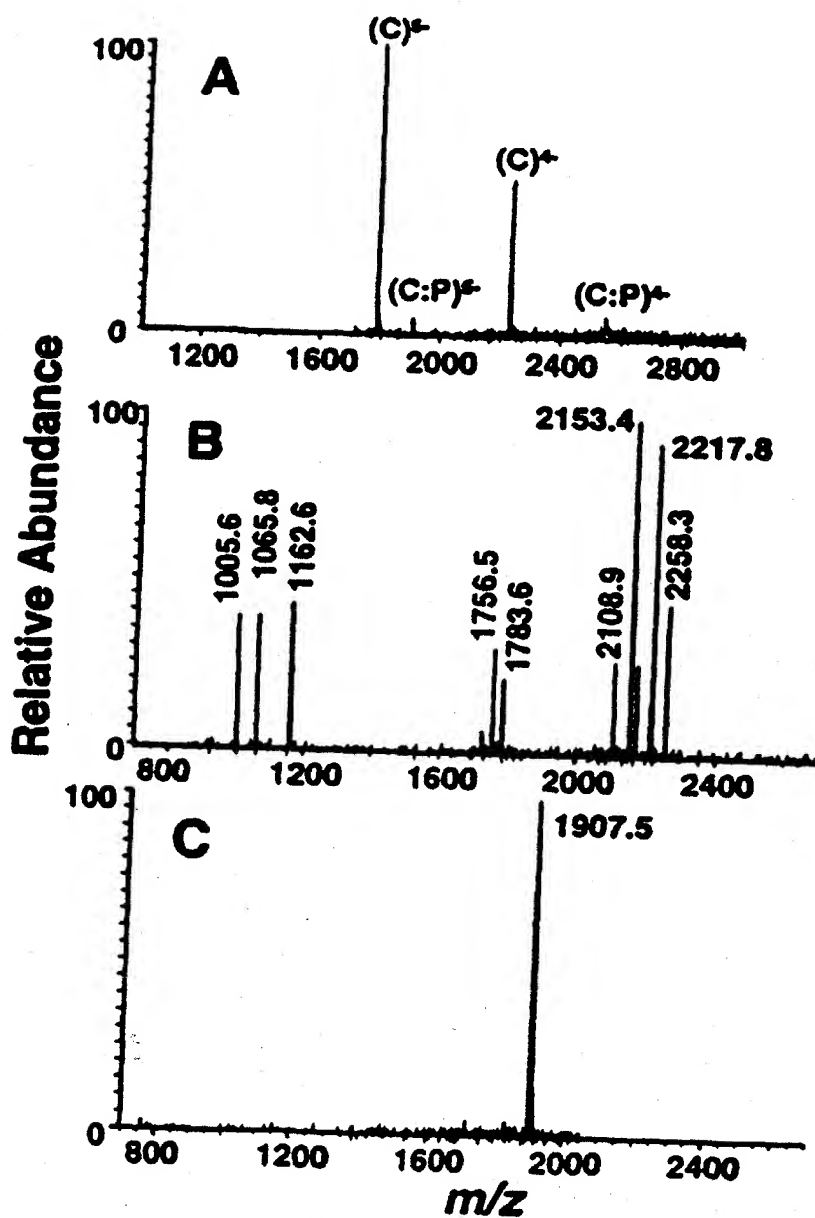
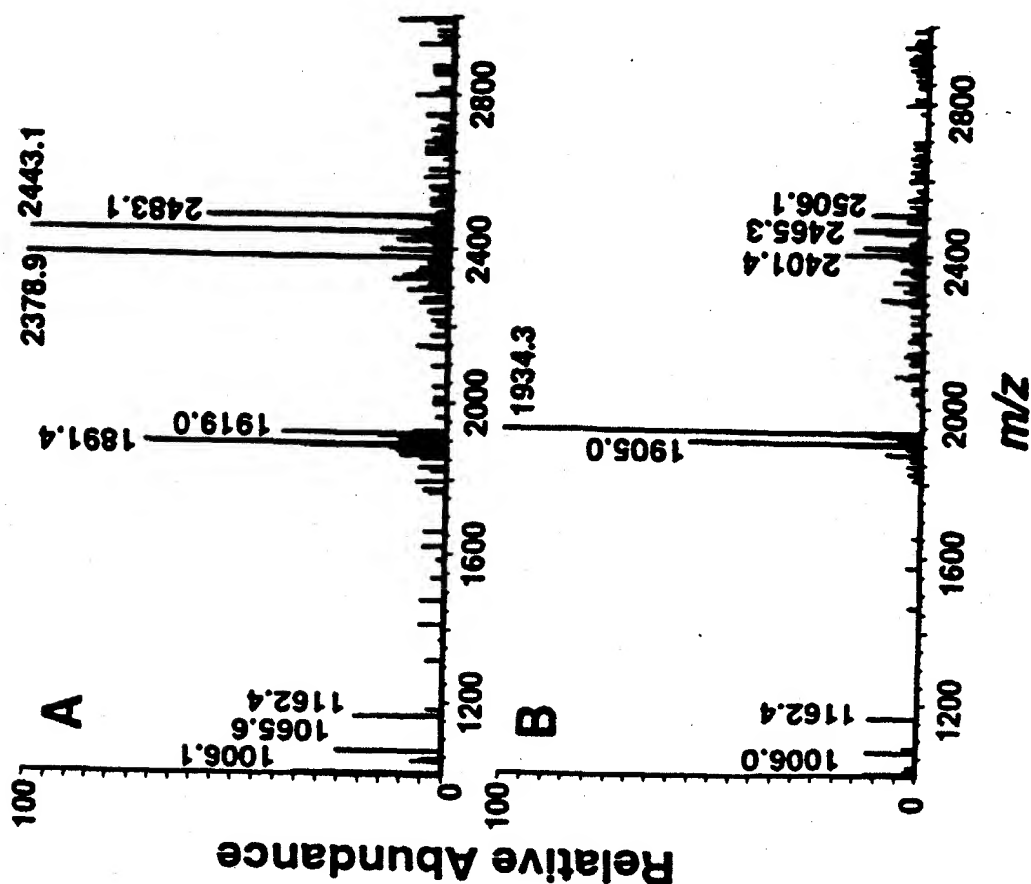
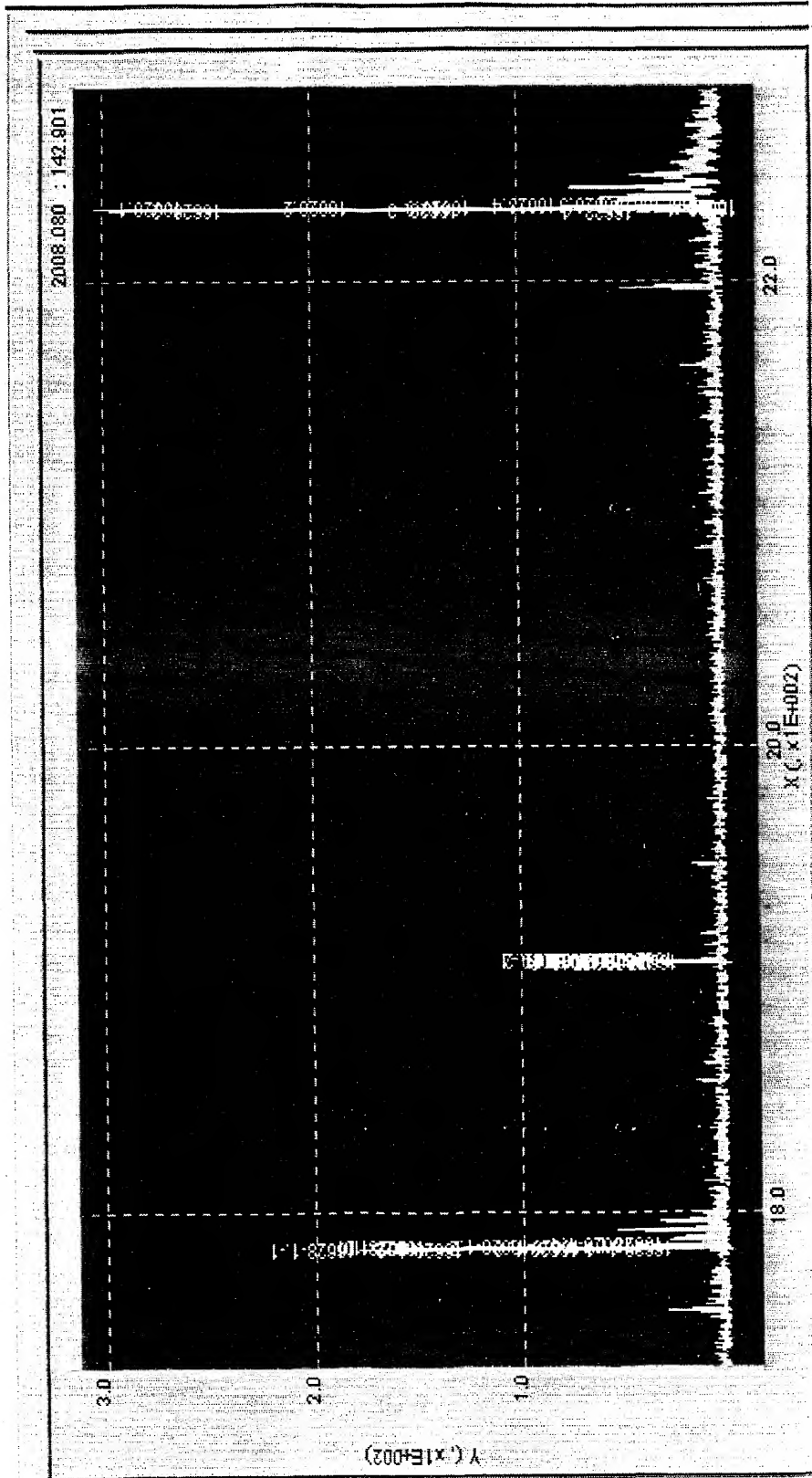


Figure 29



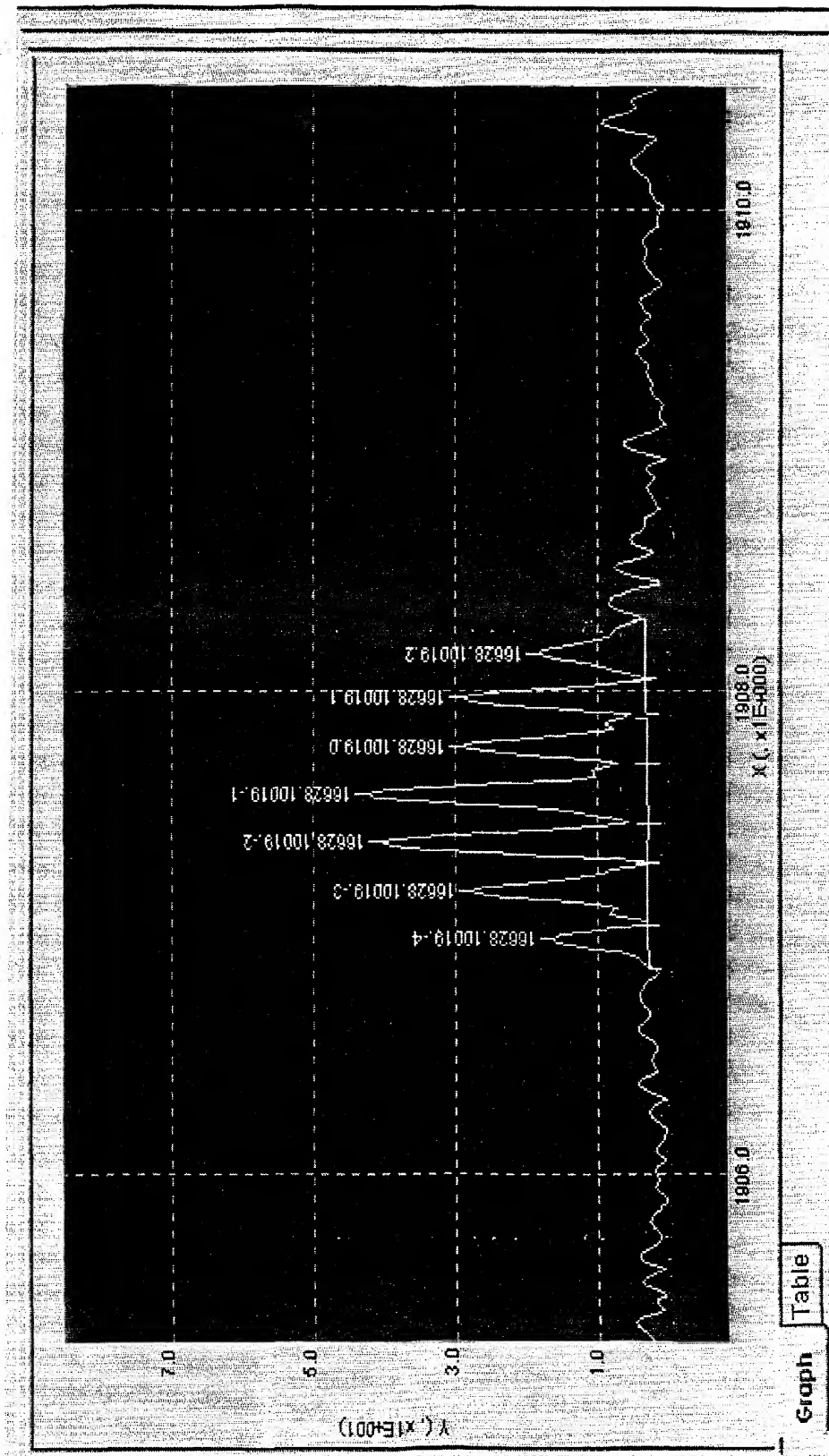
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Figure 30



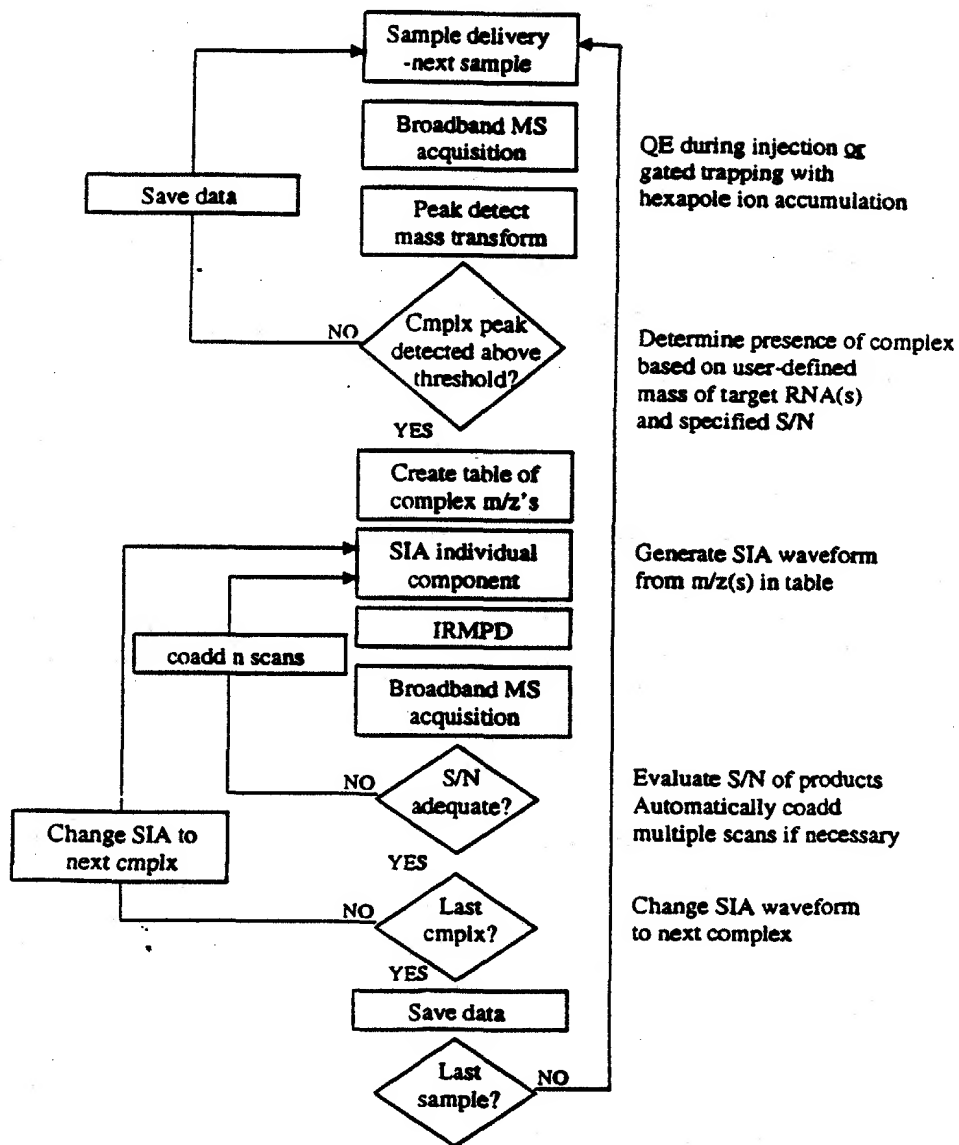
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Figure 31



nr	name	apex	start	stop	height	area
1	16628-1.-4	1783.710	1783.635	1783.834	14.55	1.63
2	16628-1.-3	1783.909	1783.834	1783.972	60.04	5.15
3	16628-1.-2	1784.109	1784.021	1784.184	115.60	11.14
4	16628-1.-1	1784.308	1784.233	1784.383	167.34	15.89
5	16628-1.0	1784.508	1784.433	1784.620	133.94	14.74
6	16628-1.1	1784.707	1784.620	1784.795	136.60	13.38
7	16628-1.2	1784.907	1784.795	1784.982	82.63	8.56
8	16628-1.3	1785.107	1785.032	1785.219	57.81	5.21
9	16628-1.4	1785.306	1785.232	1785.369	32.31	2.65
10	16628-1.5	1785.506	1785.456	1785.569	17.67	1.12
11	16628.10019.-4	1906.974	1906.874	1907.031	12.63	1.00
12	16628.10019.-3	1907.173	1907.045	1907.273	22.54	2.11
13	16628.10019.-2	1907.373	1907.287	1907.444	33.86	2.91
14	16628.10019.-1	1907.572	1907.458	1907.701	34.87	3.30
15	16628.10019.0	1907.772	1907.701	1907.843	20.93	1.55
16	16628.10019.1	1907.972	1907.900	1908.043	21.03	1.55
17	16628.10019.2	1908.157	1908.086	1908.271	10.97	0.90
18	16628.-4	2229.874	2229.679	2230.029	27.51	4.87
19	16628.-3	2230.146	2230.029	2230.263	111.72	16.23
20	16628.-2	2230.380	2230.263	2230.516	225.18	32.39
21	16628.-1	2230.633	2230.516	2230.770	280.66	40.90
22	16628.0	2230.887	2230.770	2231.023	287.24	41.95
23	16628.1	2231.140	2231.023	2231.257	242.23	34.17

Figure 33



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